IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Peled et al.	CONF NO.:	9770
SERIAL NUMBER:	10/774,843	EXAMINER :	Maria Gomez Leavitt
FILING DATE:	February 9, 2004	ART UNIT :	1633
FOR:	EXPANSION OF RENEWABLE STEM CELL POPULATIONS		

Via EFS
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DR. TONY PELED UNDER 37 C.F.R. §1.132

I, Tony Peled, declare and state that:

1. I received a Ph.D. degree from the Hebrew University - Hadassah Medical School in Jerusalem, Israel. I am the Chief Scientist, Vice President and co-founder of Gamida Cell Ltd. of Jerusalem, Israel (the Assignee of the above-referenced application). A principal aspect of my research is the study of stem cell culture and the therapeutic application of stem cell technology. I am the author of numerous peer-reviewed publications and posters, most of which are directed to cell expansion and cell differentiation, with specific focus on hematopoietic stem cells.

2. I have reviewed the Final Office Action dated April 30, 2010. I understand that claims 401, 411, 414, 416, 419, 422-424, 464, 465, 469-471 and 478-480 are rejected under 35 U.S.C. § 103(a) as being unpatentable over US Patent Publication No. 2002/0159984 to Brown ("Brown") over U.S. Patent No. 6,413,772 to Block ("Block").

3. I have reviewed the accompanying amendment and the above-referenced application in conjunction with the cited references.

4. I assert that CD34+ hematopoietic stem cells of the instant invention are cultured *ex-vivo* under conditions allowing for cell proliferation. These conditions require providing

nutrients, serum, and a combination of cytokines including each of stem cell factor, thrombopoietin, FLt3 ligand and IL-6. The CD34+ hematopoietic stem cells of the present invention can also be cultured under these conditions and further include IL-3.

5. The data accompanying this declaration shows the effect of nicotinamide (NAM) in $CD34^+$ cells derived from umbilical cord blood during 3 weeks in cultures supplemented with cytokines (each of FLT3, IL-6, TPO, and SCF). Analysis included the number of total nucleated cells (TNC), colony forming unit cells (CFUc) and phenotypic characterization of hematopoietic progenitors, $CD34^+$ and $CD34^+CD38^-$ cells.

6. Figure 1 accompanying this declaration shows cord blood-derived purified $CD34^+$ cells cultured for 3 weeks with cytokines (each of FLT3, IL-6, TPO, and SCF) or with cytokines (each of FLT3, IL-6, TPO, and SCF) and nicotinamide ("NAM") at 2.5 and 5 mM. Each bar represents the average ± SE of 4 independent experiments.

7. Panel A-E shows the analysis of cultured cells one week post seeding.

8. Panel A shows the number of total nuclear cells (TNC) (*$P<0.01$ vs. NAM, 2.5 and 5 mM).

9. Panel B shows the number of $CD34^+$ cells (*$P<0.008$ vs. NAM).

10. Panel C shows representative FACS analysis dot plots of cells double stained with CD34 PE and CD38 FITC.

11. Panel D shows percentages of $CD34^+CD38^-$ cells (*$P<0.01$ vs. NAM non-treated cultures).

12. Panel E shows the numbers of $CD34^+CD38^-$ cells (*$P<0.03$ vs. NAM non-treated cultures).

13. To track cell division history, freshly purified $CD34^+$ cells were labeled with PKH2, cultured and analyzed 7 day post seeding. Histograms of PKH fluorescence intensity of $CD34^+$ (Panel F) and $CD34^+CD38^-$ (Panel G) cells are shown. The histograms present the same number of cells for both control and NAM-treated cells in a representative experiment out of three experiments performed.

14. Panel H shows the median fluorescence intensities of NAM expanded cells on day 7 cultures of three separate experiments as percentages of control cultures treated with cytokines alone.

15. Panel I shows the percentages of $CD34^{+}CD38^{-}$ cells, one, two and three weeks post seeding (*$P \leq 0.01$ vs. NAM non-treated cultures). Panels J-L show the fold expansion (FE) of TNC (*$P<0.01$ vs. NAM, **$P<0.03$ vs. NAM 5mM) (J), CFUc (*$P<0.03$ vs. NAM, **$P<0.02$ (K) and $CD34^{+}$ cells (*$P \leq 0.01$ vs. NAM) (L), one, two and three weeks post seeding.

16. Figure 2 accompanying this declaration shows cord blood derived purified CD34+ cells cultured with cytokines, with and without NAM (2.5-5mM) and FACS analyzed after 3 weeks as following: cells were count and thereafter stained with FITC-conjugated antibodies against differentiation antigens (CD38, CD33, CD14, CD15, CD3, CD61, CD19) and with PE-conjugated antibodies against CD34. Positive PE and negative FITC cells were considered as CD34+Lin- cells. The results show the actual number in culture of CD34+CD38- Lin- cells calculated from the total number of cells. Each experiment was repeated with cells derived from 6 different cord blood units.

17. The data accompanying this declaration shows, as early as one week post seeding, that the number of total nuclear cells (TNC) (Fig. 1A) and $CD34^{+}$ cells (Fig. 1B) were substantially lower, while percentages (Fig.1C-D) and absolute number (Fig. 1E) of $CD34^{+}CD38^{-}$ cells were significantly higher in cultures treated with 2.5 and 5 mM NAM as compared with control cultures treated with cytokines only. The divisional history of seeded $CD34^{+}$ cells stained with PKH indicated that during the first week in culture, the vast majority of $CD34^{+}$ cells underwent several cycles under both culture conditions (Fig. 1F-G), with consistent lesser divisions (higher fluorescence intensity) of cells cultured with NAM (Fig. 1H). Slower cycling was particularly prominent in the $CD34^{+}CD38^{-}$ subset (Fig. 1G) which, nevertheless, increased within the expanded cell population from week one through week-3 (Fig.1I). After three weeks in culture, fold expansion of TNC (Fig. 1J), CFUc (Fig. 1K) and $CD34^{+}$ cells (Fig. 1L) in NAM-treated reached the values in NAM non-treated cultures. Phenotype characterization of lineage differentiated cells in three week cultures revealed lessening of differentiation in cultures treated with NAM (2.5 and 5 mM) than in cultures treated with

cytokines alone, as demonstrated by significant lower percentages of CD14, CD11b, CD11c and $CD15^+$ cells (Fig. 2).

18. Thus, it is shown that $CD34^+$ cells cultured with NAM displayed an initial slower proliferation rate than their counterparts cultured without NAM. This was most pronounced in the putative HPC compartment defined as $CD34^+CD38^-$ cells, along with an increase in their numbers in the presence of NAM. Our results demonstrate that culturing with each of FLT3, IL-6, TPO, and SCF in the presence of NAM produced an expanded CD34+ hematopoietic stem cell population with an increased proportion of CD34+/Lin- and CD34+/CD38- cells in the expanded culture as compared to CD34+ cells cultured in the presence of those cytokines and nutrients without exogenously added nicotinamide.

19. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

A. P.
Tony Peled, Ph.D.

Signed this 30 day of July, 2010

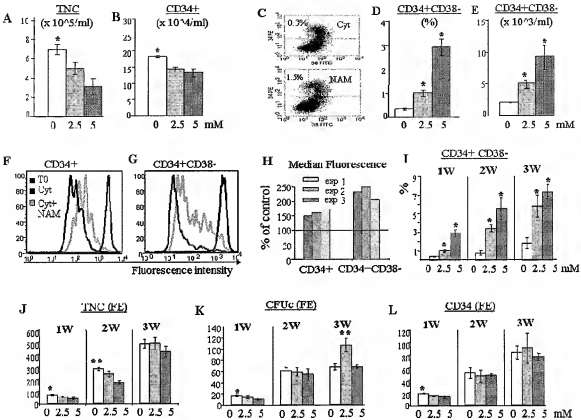

Fig.1

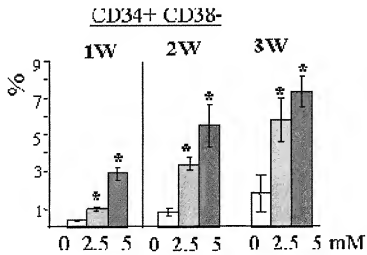

Figure 1

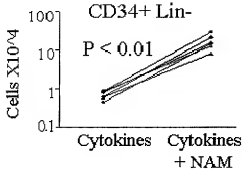

Figure 2

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